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Short communication

Influence of different factors on the inactivation of *Salmonella senftenberg* by pulsed electric fields

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Abstract

The influence of growth phase, cell concentration, pH and conductivity of treatment medium on the inactivation of *Salmonella senftenberg* by high electric field pulses (HELP) was studied. Cells were more resistant to HELP treatments at the beginning of the logarithmic phase and at the stationary phase. Microbial inactivation was not a function of the initial cell concentration. At constant input voltage, electric field strength obtained in the treatment chamber depended on medium conductivity. At the same electric field strength, conductivity did not influence *S. senftenberg* inactivation. At the same conductivity, inactivation of *S. senftenberg* was bigger at neutral than acidic pH.

Keywords: Salmonella senftenberg; High electric field pulses; pH; Conductivity; Growth phase

1. Introduction

High electric field pulses (HELP) as a non-thermal process of food preservation are under intense study because of its potential of inactivating microorganisms without altering organoleptic and nutritional qualities of foods (Barbosa-Cánovas et al., 1998). The process consists of the application of short duration (1 to 100 μs) high electric field pulses (10–50 kV/cm) to a food placed between two electrodes.

In this investigation, the influence of growth phase, cell concentration, pH and conductivity of treatment medium on the inactivation of *Salmonella senftenberg* by HELP treatments was studied.

Microbial resistance to physico-chemical agents is influenced by many factors (Tomlins and Ordal, 1976). These factors include the intrinsic resistance of the microorganisms, the physiological state of the microorganisms and the characteristics of the treatment medium. The influence of these factors on the microbial inactivation by HELP treatments is not well known. The design of effective HELP treatments that assure the safety and stability of foods require identification of the critical parameters of the

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process and to know the factors that affect HELP resistance of microorganisms.

In this investigation, the influence of growth phase, cell concentration, pH and conductivity of treatment medium on the inactivation of *Salmonella senftenberg* by HELP treatments was studied.

2. Materials and methods

2.1. Microorganism

The strain of *Salmonella senftenberg* 775W (ATCC 43845) used in this investigation was supplied by the Spanish Type Culture Collection. The microbial suspension was prepared by adding to 50 ml of nutrient broth (Biolife, Milan, Italy) an overnight broth subculture to a final concentration of 10^6 cells/ml. The culture was then incubated at 37°C. To study the influence of the cell age on the resistance to HELP treatments after inoculation, 1-ml samples were taken at preset intervals and cell concentration and HELP resistance were measured. The other experiments described were performed with cells at the stationary growth phase.

Before treatment, microorganisms were centrifuged at $6000 \times g$ for 5 min and resuspended in the corresponding treatment medium.

After treatment, appropriate serial dilutions were prepared in sterile 0.1% peptone water and plated onto nutrient agar. The number of surviving organisms was enumerated after incubation for 24 h at 37°C.

2.2. HELP treatments

High electric field pulses were produced by discharging a 2-μF capacitor (Maxwell, San Diego, CA) via an IGBT switch (Behlke, HTS 101-80-FI, Frankfurt, Germany) into a treatment chamber. A cylindrical plastic tube closed with two polished stainless steel cylinders was used as treatment chamber. Distance between electrodes was 0.25 cm and electrode area was 2.01 cm^2. The circuit configuration generated square waveform pulses at different frequencies and pulse widths. A pulse width of 2 μs and a pulse repetition rate of 2 Hz was used in this study. Actual electric field strength applied was measured in the treatment chamber with a high voltage electrode connected to an oscilloscope (Tektronix, TDS 220, Wilsonville, OR).

To study the influence of the conductivity on the microbial inactivation, cells were suspended in McIlvaine citrate–phosphate buffer (Dawson et al., 1974) of pH 7.0 diluted at different concentrations to get different conductivities. To study the influence of pH, cells were suspended in different buffers (McIlvaine citrate–phosphate buffer, phosphate buffer, citric–citrate buffer and Miller and Golder buffer) (Dawson et al., 1974) of pH 3.8 and 7.0 diluted to get the same conductivity (2 mS/cm).

The temperature of the samples after treatment was always lower than 35°C.

3. Results and discussion

Fig. 1 shows the growth curve of *Salmonella senftenberg* at 37°C and the influence of the cell age on the HELP resistance of this microorganism. A HELP treatment of 200 pulses at 19 kV/cm was applied to the cells at different growth stages. At the stationary phase and the beginning of the logarithmic phase, cells were more resistant to the electric field treatment than cells in the logarithmic phase. However, the influence of the cell age was not very important. The maximum difference in the number

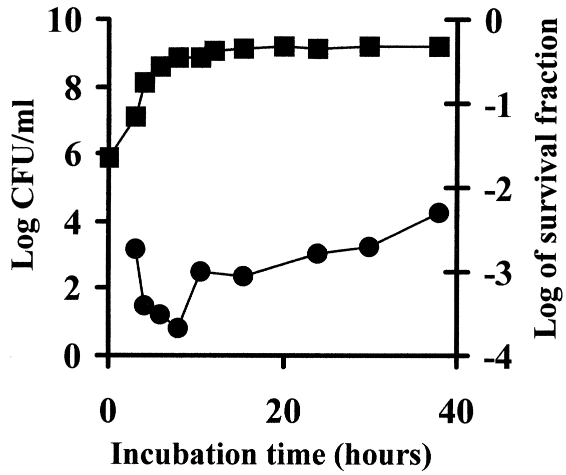

Fig. 1. Growth curve at 37°C (■) and influence of cell age on the HELP resistance (●) of *S. senftenberg*. Treatment conditions: McIlvaine citrate–phosphate buffer of pH 7 and 2 mS/cm; pulse width, 2 μs; frequency, 2 Hz.

of survivors between the most and the less resistant cells was only 1.5 log cycles. Similar results have been observed by Pothakamury et al. (1996) with *Escherichia coli* but in this case the difference between the survival fraction of the most and the less resistant cells was two log cycles.

To study the influence of the cell concentration on the microbial inactivation by HELP treatments, initial concentration of *Salmonella senftenberg* was set at 3×10^9, 3×10^7 and 3×10^5 CFU/ml and subjected to a treatment at 22 kV/cm (Fig. 2). Results obtained were similar to results of other authors (Zhang et al., 1995) and indicated that the microbial inactivation is not a function of the initial concentration. However, these results disagree with the influence of cell concentration on the survival rate of *E. coli* observed by Hülsheger and Niemann (1980).

The inactivation of *S. senftenberg* in pH 7.0 McIlvaine citrate–phosphate buffer of different conductivities is shown in Fig. 3. At the same input voltage (7 kV), the electric field strength applied to the sample and the *Salmonella* inactivation were lower at higher conductivities (Fig. 3A). However, when input voltage was modified to obtain the same electric field strength (22 kV/cm) at the different conductivities the conductivity did not influence the inactivation of *Salmonella* (Fig. 3B).

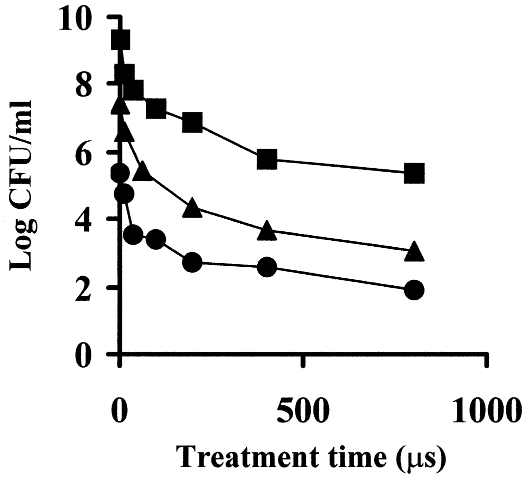

Fig. 2. Influence of initial cell concentration on the *S. senftenberg* inactivation by HELP treatments. Treatment conditions: 3×10^9 CFU/ml (■), 3×10^7 CFU/ml (▲), 3×10^5 CFU/ml (●); McIlvaine citrate–phosphate buffer of pH 7 and 2 mS/cm; pulse width, 2 μs; frequency, 2 Hz.

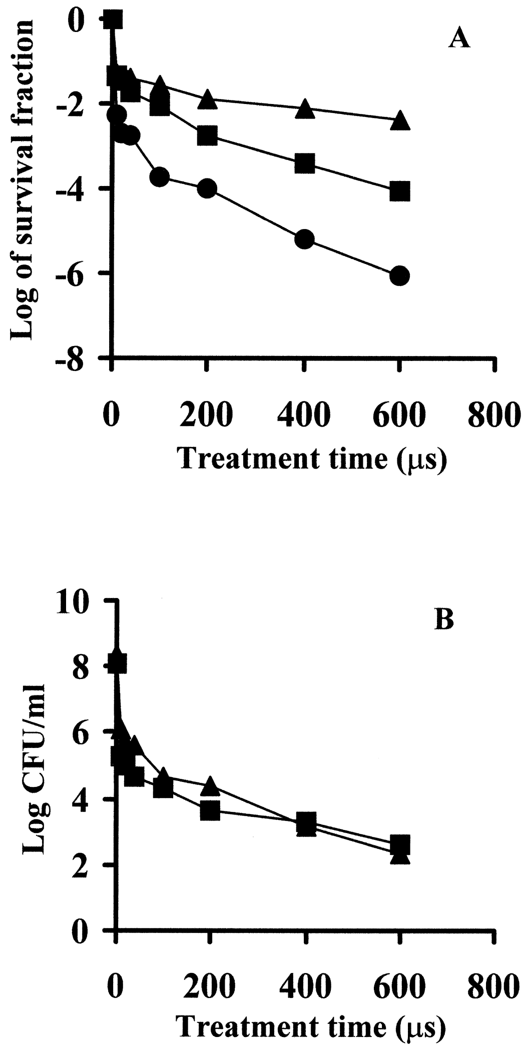

Fig. 3. Influence of conductivity on the *S. senftenberg* inactivation by HELP treatments. (A) Inactivation of *S. senftenberg* in media of different conductivities at a constant input voltage (7 kV): 2 mS/cm, 22 kV/cm (●); 3 mS/cm, 20 kV/cm (■); 4 mS/cm, 18 kV/cm (▲). (B) Inactivation of *S. senftenberg* in media of different conductivities at a constant electric field strength (22 kV/cm): 2 mS/cm, 7 kV (▲); 3 mS/cm, 7.8 kV/cm (■). Treatment conditions: pulse width, 2 μs; frequency, 2 Hz.

The influence of the pH on the microbial inactivation by HELP treatments is unclear. Several authors (Sale and Hamilton, 1967; Hülsheger et al., 1981) reported that there was no influence of the pH, however Vega-Mercado et al. (1996) found that the inactivation of *E. coli* by HELP treatments was more significant at pH 5.6 than 6.8. We observed that at

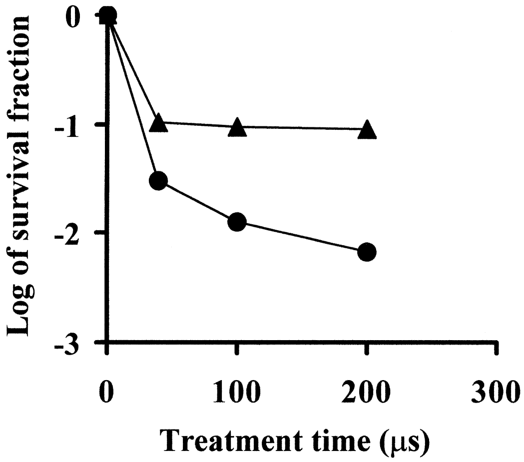

Fig. 4. Influence of pH on the *S. senftenberg* inactivation by HELP treatments. Treatment conditions: McIlvaine buffer of pH 7 (●) and 3.8 (▲); 2 mS/cm; 22 kV/cm; pulse width, 2 μs; frequency, 2 Hz.

the same conductivity (2 mS/cm) *Salmonella* was more resistant in McIlvaine buffer of pH 3.8 than 7.0 (Fig. 4). To check if the highest resistance at lower pH could be due to the composition of McIlvaine buffer that protect the cells against the HELP treatment, we performed the same experiment with different buffers (data not shown).

Results obtained show that *Salmonella* was always more resistant at acidic pH and at the same pH the buffer composition did not affect the inactivation of *Salmonella*.

4. Conclusion

Results obtained in this investigation indicated that the microbial inactivation by HELP treatments depends on many parameters. As the influence of the parameters can depend on the microorganisms, many studies are needed to improve and optimise the HELP treatment as a non-thermal process of food preservation.

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